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Efficacy of 35% PEROX-AID® (Hydrogen Peroxide) to Control Infestations of *Gyrodactylus salmonis* in Freshwater-reared Rainbow Trout *Oncorhynchus mykiss*

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Gyrodactylus are monogenean parasites, nearly all of which have a direct life cycle requiring no intermediate host. The genus *Gyrodactylus* comprises 409 recognized species. Approximately 60% of these are fish host-specific, while others can infect multiple fish species (Bakke et al. 2007). One species of particular interest to salmonid culturists in the United States is *Gyrodactylus salmonis*, which attaches to and deeply penetrates the epidermis of its host. The parasite feeds primarily on epidermal and mucus cells and occasionally on dermal cells, thus facilitating secondary infections (Cone and Odense 1984). *G. salmonis* infestations occur most frequently during winter and early spring when water temperatures are $\leq 8^{\circ}\text{C}$ (Beverly-Burton 1994). *Gyrodactylus* infestations also occur in fall as water temperature declines (Bakke et al. 2007). The primary behavioral clinical sign is flashing. Other gross clinical signs include erosion of fins, pale or discolored flanks, thickened cuticle, obvious secretions of mucus, and emaciation. Some authors have also noted epidermal thinning and decreased mucus production (Wells and Cone 1990). During *G. salmonis* infestations, fish culturists can often minimize mortality by improving environmental rearing conditions, administering chemotherapeutic bath treatments, or both.

A number of topical sanitizing agents, including hydrogen peroxide (H_2O_2), have historically been used and tested for the control of ectoparasite infestations on fish and fish eggs. In January 2007, 35% PEROX-AID® (35% active H_2O_2 ; Eka Chemicals, Inc., Marietta, GA) was approved by the U.S. Food and Drug Administration (FDA) for the following indications: use as an external microbicide for control of mortality in (1) freshwater-reared finfish eggs due to saprolegniasis, (2) freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium branchiophilum*, and (3) freshwater-reared coolwater finfish and channel catfish due to external columnaris disease associated with *F. columnare*. Currently, this product is not approved to control or reduce infestations of ectoparasites. To generate data needed to expand the current 35% PEROX-AID® label claim to allow for its use as a parasiticide, data are needed to demonstrate efficacy against a specific ectoparasite. As such, a trial was conducted to evaluate the effectiveness of H_2O_2 to control infestations of *G. salmonis* in freshwater-reared salmonids.

Methods

The trial was conducted in October 2010 at the U.S. Fish and Wildlife Service's Ennis National Fish Hatchery (NFH; Ennis, Montana USA). The test article was 35% PEROX-AID® (35% active H_2O_2). Test fish were adult rainbow trout (mean length, 45.6 cm; mean weight, 1.3 kg) impartially collected from a reference population infested with *G. salmonis*. The trial was conducted under an FDA-accepted research study protocol. Treatment objectives were to (a) show a significant difference in mean abundance of *G. salmonis* between treated and control groups, and (b) achieve a 90% reduction in mean abundance of *G. salmonis*. Both objectives had to be met to demonstrate treatment efficacy. Completely randomized design procedures were used to allocate test fish ($N = 20$ fish per tank) and treatment conditions to six test tanks (three treated and three control). Test-tank rearing volume was 1,464 L; thus, loading density in each tank was 17.8 g per L.

The trial comprised a 3-d acclimation period, 3-d treatment period, and 7-d posttreatment period. During the acclimation period, 30 fish were impartially collected from the reference population to estimate baseline prevalence and mean abundance of *G. salmonis*. A skin scrape was taken from the right side of each fish with the sharp edge of a scalpel blade (width of blade ~ 1 cm) beginning at the anterior attachment point of the dorsal fin and proceeding posteriorly for approximately 5 cm. The skin scrape was smeared onto a clean microscope slide along with a drop of hatchery water, cover-slipped, and examined with a compound microscope at $40\times$ magnification. All parasites on the slide were counted. Twenty of the 30 fish were randomly sampled and necropsied to establish baseline fish health conditions. During the treatment period, 35% PEROX-AID® was administered to treated tanks as a static bath at a target concentration of 50 mg H_2O_2 per L for 30 min per d on two alternate days. A sham treatment of hatchery water was administered to control tanks. Ten fish were sampled to estimate *G. salmonis* infestation level on posttreatment days 2 and 7. A skin scrape was taken, prepared, and evaluated from each fish as described above. Dorsal fins were hole-punched to identify fish sampled at 2 d posttreatment, and these fish were returned to their respective tanks to maintain fish-loading densities. Fish sampled on posttreatment day 2 were not sampled on posttreatment day 7.

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Mortality, general fish behavior, fish-feeding behavior, water temperature, and dissolved oxygen concentration data were collected daily during the trial. Source water hardness, alkalinity, and pH were measured twice during the trial (treatment day 1 and posttreatment day 7). Water samples were collected 15 min into each treatment from two randomly selected tanks (one treated and one control) on each treatment day and analyzed to determine hydrogen peroxide concentration. The titrimetric method described by Jeffery et al. (1989) was used to verify H₂O₂ concentrations in the water samples.

Treatment efficacy was assessed at 2 d and 7d posttreatment. Mean abundance of *G. salmonis* was compared between treatment groups with a mixed-model, nested analysis of variance (ANOVA; $P < 0.05$). To compensate for parasite counts of zero in some fish, the count for each fish was increased by one and log_e-transformed before analysis. The least squares means from the ANOVA were back-transformed ($e^{\text{treatment group mean}}$) to geometric means, which were then used to calculate percent reduction in mean abundance:

$$\text{Percent reduction} = 100 - \left[100 \times \frac{(\text{geometric mean}_{\text{treated}} - 1)}{(\text{geometric mean}_{\text{control}} - 1)} \right]$$

Results and Discussion

At 2 d posttreatment, mean number of *G. salmonis* counted per fish (0.1 ± 0.3) on the 10 fish sampled from treated tanks was significantly ($P < 0.001$) different from that in control tanks (34.4 ± 43.2). Percent reduction in mean abundance was 99.4%. At the end of the trial (7 d posttreatment), mean number of *G. salmonis* per fish on the 10 fish not previously sampled in treated tanks (0.1 ± 0.3) was also significantly different ($P < 0.001$) from that in control tanks (38.5 ± 77.4). Percent reduction in mean abundance was 99.7%.

Infestation prevalence in the reference population fish sampled before the trial started was 100%. Mean prevalence in treated tanks decreased to 13% (range, 10 - 20%) at 2 d posttreatment and 7% (range, 0 - 10%) at 7 d posttreatment. Conversely, mean prevalence of *G. salmonis* in control tanks remained high and was 93% (range, 90 - 100%) at 2 d posttreatment and 100% at 7 d posttreatment.

No fish died during the trial. General fish behavior was characterized as normal in both treated and control tanks, and all fish fed aggressively.

During the trial, mean water temperature was 12.0°C (range, 12.0 - 12.1°C), and mean dissolved oxygen concentration was 7.2 mg per L (range, 6.8 - 7.7 mg/L). Mean source water hardness, alkalinity, and pH were 254 mg per L (as CaCO₃),

146 mg per L (as CaCO₃), and 8.0, respectively. All water quality parameters were considered suitable for rearing healthy rainbow trout.

The mean concentration of H₂O₂ administered to the two treated tanks that were sampled was 57.9 mg per L (+15.9% of target dose; 58.5 mg per L and 57.4 mg per L). Hydrogen peroxide was not detected in the control tanks.

Based on these results, we concluded that 35% PEROX-AID[®] administered at a target dosage of 50 mg H₂O₂ per L as a static bath for 30 min per d on two alternate days was effective in controlling infestations of *G. salmonis* in adult rainbow trout. Results have been submitted to FDA/CVM and will be used to support expanding the current use of 35% PEROX-AID[®] to include use as a parasiticide on freshwater-reared salmonids.

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